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Radiation Resistance of Spores of Some *Clostridium perfringens* Strains

WALTER J. CLIFFORD[1] AND ABE ANELLIS*

Food Sciences Laboratory, U.S. Army Natick Laboratories, Natick, Massachusetts 01760

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Clostridium perfringens spores (eight strains) were irradiated in a model system with ^{60}Co gamma rays at −30 C. The quantal response data obtained were analyzed with extreme value statistics. It was found (at the 95% confidence level) that all eight strains followed the same rate of death and that this rate was probably (at the 95% level) not exponential. The statistics did not exclude, however, a normal, lognormal, Weibull, or related rate of spore kill. A more definitive study would be required to distinguish between the latter distributions.

Spores of 12 strains of *Clostridium perfringens* (obtained from C. L. Duncan, Food Research Institute, Madison, Wis.) have previously been produced in a biphasic glucose-ion-exchange resin medium (4). It was therefore of interest to determine their radiation resistances in a model system. We selected six of seven strains which yielded significantly more spores by our method (4) than by the popular Duncan and Strong technique (7), one of three strains which formed fewer spores, and one of two strains which elicited the same number of spores. These eight strains had been isolated from various foods.

The spores, suspended in Sorensen phosphate buffer (0.067 M, pH 7.0), were heat-shocked (65 C for 30 min) and enumerated in triplicate plates with Trypticase-yeast extract-glucose-sulfite-iron agar as described earlier (5). Ice-chilled spore suspensions (10^5/ml) were then distributed aseptically in 1.0-ml quantities into sterile cotton-plugged Pyrex tubes (10 by 75 mm), vacuum sealed (125 mm of Hg) in metal cans (401 by 411), and frozen to −35 ± 5 C; the handling procedure was detailed elsewhere (1). Duplicate cans (20 replicate tubes) were irradiated with ^{60}Co gamma rays at −30 ± 5 C in the dose range 0.7 to 2.5 Mrad in units of 0.1 Mrad; the dose rate was 47 Krad/min and the transient dose was 40 Krad. The irradiated tubes were thawed (3 ± 1 C) overnight, and 1.0 ml of double-strength Trypticase-yeast extract-glucose broth (boiled and cooled to remove oxygen) was added aseptically to each tube; the iron citrate and sodium sulfite were omitted from the medium because their possible inhibitory effects on the recovery and/or proliferation of the increasingly radiation-damaged spores were unknown. The tubes were then heat-sealed in an oxygen-gas flame, incubated for 8 weeks at 30 ± 2 C, and observed for turbidity. The entire contents of each tube were then subcultured into Trypticase soy broth (BBL) and incubated at 30 ± 2 C up to 7 days; samples showing good growth at any time within this period were verified as *C. perfringens* by biochemical (mannitol, lactose, nitrate) and morphological (motility, microscopic) characteristics.

The above experiment simulated an inoculated pack study by providing "partial spoilage," or quantal response, data in a model system (Table 1). Since the rate of spore kill in this type of system has not yet been adequately elucidated, these results were estimated as "12D" doses (mandatory minimal radiation doses for a microbiologically safe food irradiation process) on the supposition that the quantal responses of the eight strains followed either the conventionally assumed exponential (2, equation 3), Weibull (2, equation 5), or normal (3) distribution (Table 2). The two latter distribution functions are non-exponential; hence their "12D" equivalents cannot be reduced to, or calculated initially as, familiar D values.

The minimal radiation dose data (Table 2) reflect the comparative order of resistances of the eight strains tested. Strain FD-7 was the most resistant, followed by FD-1, if the rate of "partial spoilage" was either exponential or Weibull; the order is reversed if the rate was normal. Strain T-65 was the most sensitive regardless of the distribution function. The computed exponential data were higher than the normal minimal radiation dose values in all

[1] Present address: Cochise Pathology Consultants, Sierra Vista, Ariz. 85635.

TABLE 1. *Radiation "partial spoilage" data of some strains of C. perfringens spores*[a]

Dose received (Mrad)	No. of tubes out of 20 showing survivors for strains[b]							
	NCTC 8238	NCTC 8798	68900	T-65	FD-1	FD-2	FD-5	FD-7
0.7	20	20	20	15	20	20	18	20
0.8	20	20	20	11	20	20	13	20
0.9	20	20	20	9	20	20	10	20
1.0	19	20	20	6	20	20	8	20
1.1	15	16	20	2	20	20	7	20
1.2	9	7	20	0	20	20	3	20
1.3	4	3	19	0	20	20	1	20
1.4	1	2	14	0	20	19	0	20
1.5	0	1	8	0	20	15	0	20
1.6	0	1	6	0	20	12	0	20
1.7	0	0	4	0	20	7	0	17
1.8	0	0	1	0	15	0	0	14
1.9		0	0	0	9	1	0	8
2.0	0	0	0	0	7	0	0	4
2.1	0	0	0	0	5	0	0	1
2.2	0	0	0	0	5	0	0	0
2.3	0	0	0	0	1	0	0	0
2.4	0	0	0	0	0	0	0	0
2.5	0	0	0	0	0	0	0	0

[a] Irradiated with ^{60}Co gamma rays at -30 ± 5 C.

[b] The initial number of spores per milliliter of sample irradiated, of each strain, was (in sequence): 4.3×10^5, 1.2×10^6, 2.6×10^5, 1.8×10^5, 7.2×10^5, 3.7×10^5, 8.9×10^4, 1.8×10^5.

instances and were also higher than the Weibull "12D" equivalents with all strains except FD-5. A rank correlation test (6) indicated that the order of strain resistance agreed best between the Weibull and normal distributions ($R = 0.95$) rather than between the exponential and Weibull ($R = 0.79$) or between the exponential and normal ($R = 0.88$).

Analysis of the quantal response data (Table 1) with extreme value statistics (14; E. W. Ross, Jr., unpublished data) indicated (at the 95% confidence level) that all eight strains followed the same distribution form, and that this common form was probably (at the 95% level) not exponential. The statistics did not exclude, however, either the normal, lognormal, extreme value, or related distributions; additional data in a model system would be required to distinguish between the latter density functions. A similar situation occurred with an inoculated beef pack containing *Clostridium botulinum* spores which had been irradiated with ^{60}Co gamma rays at -30 C (A. Anellis et al., manuscript in preparation). Analysis of the latter partial spoilage data with extreme value statistics indicated (with 90% confidence) that the rate of spore death was not a simple exponential distribution, but could be a normal, lognormal, Weibull, or a shifted exponential.

Previous results on the radiation resistance of *C. perfringens* spores were obtained from dose-survival curves which yielded 3 to 6 log reductions; some of these plots contained an initial shoulder (8, 12, 13) and others did not (9–11). Since all of these observations were reported as "D" values, we could not compare the exponentially extrapolated "12D" values with our non-exponential findings.

TABLE 2. *Comparative radiation resistances of some strains of C. perfringens spores*[a]

Strain	Method of computing a 12D dose (Mrad)		
	Exponential[b]	Weibull[c]	Normal[d]
NCTC 8238	2.73	1.86	1.90
NCTC 8798	2.58	2.00	2.00
68900	3.13	2.98	2.42
T-65	1.93	1.58	1.78
FD-1	3.91	3.30	3.04
FD-2	3.39	2.11	2.43
FD-5	2.23	2.70	2.19
FD-7	4.14	3.46	2.60

[a] Irradiated with ^{60}Co gamma rays at -30 ± 5 C.

[b] See reference 2, equation 3.

[c] See reference 2, equation 5.

[d] See reference 3.

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